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Source: Journal of Wildlife Diseases, 26(1) : 107-111

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.1.107

Journal of Wildlife Diseases, 26(1), 1990, pp. 107–111

Serologic Survey of Selected Zoonotic Disease Agents in Black-Tailed Jack Rabbits from Western Texas

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ABSTRACT: A serologic survey for the agents of Rocky Mountain spotted fever (RMSF) (*Rickettsia rickettsii*), *Borrelia* spp. including the causative agent for Lyme disease (*Borrelia burgdorferi*), and plague (*Yersinia pestis*) was conducted on blood samples collected from 30 and 46 black-tailed jack rabbits (*Lepus californicus*) from an urban environment in Lubbock, Texas (USA) during winter 1987 and the following spring 1988, respectively. Antibody titers to the agents of RMSF and borreliosis were detected in sera of 28 and 1% of the jack rabbits, respectively. Neither organisms (rickettsiae and/or spirochetes) nor their associated antigens were detected in any of the tissue or whole blood samples; plague antibodies were not detected in the 76 jack rabbits sampled. Four of 18 ticks (*Dermacentor parumapertus*) removed from 12 jack rabbits were positive for RMSF using the fluorescent antibody test. The black-tailed jack rabbit is a common wildlife species living in close proximity to higher density human populations in many areas of the southwestern United States. Our results indicate the potential importance of urban populations of this mammal as reservoirs for at least one important zoonotic disease, RMSF, in western Texas.

Key words: *Borrelia* spp., borreliosis, black-tailed jack rabbit, *Lepus californicus*, Lyme disease, *Rickettsia rickettsii*, Rocky Mountain spotted fever, sylvatic plague, serologic survey, urban reservoir, *Yersinia pestis*.

The black-tailed jack rabbit (*Lepus californicus*) occurs throughout the southwestern United States; it is a common and highly adaptable species with sizable urban populations living in close proximity to the higher density human populations throughout its range (Chapman and Willner, 1986). Although it has been implicated as a reservoir for certain arthropod-transmitted zoonoses (Parker et al., 1939, 1951; Philip et al., 1955), there has been little emphasis placed on the importance of this mammal as a reservoir for important zoonoses in the urban environment. Thus, the objective of the present study was to determine the prevalence of certain selected zoonotic disease agents and their associated arthropod vectors in an urban locality in western Texas.

Thirty and 46 black-tailed jack rabbits were live-trapped during 1 to 7 December 1987 and 30 April to 8 May 1988, respectively, on the Texas Tech University campus (Lubbock, Texas; 33°35′N, 101°54′W). Jack rabbits were placed in gunney sacks, transported to a holding area and euthanized with a 1.5 ml intraperitoneal injection of T61 euthanasia solution (Taylor Pharmaceuticals, Decatur, Illinois 62525, USA). Two blood samples were taken via a heart puncture with a 12 cc syringe and an 18 ga needle. One whole blood sample containing EDTA was stored on wet ice while the other blood sample was centrifuged at 1,400 rpm for 15 min, and its serum was collected and stored on wet ice. Thick blood smears were prepared by placing one drop of whole blood on a microscopic slide and laking it with a wooden applicator stick until dry. The presence of antibodies to sylvatic plague was tested with the Nobuto Strip Type I paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The absorbing portion of the Nobuto paper was immersed in blood, removed and allowed to air dry at room temperature.

Immediately following the collection of blood samples, jack rabbits were placed in a white procelain closed container with ether-soaked gauze to anesthetize the arthropod parasites. Ectoparasites were collected directly from jack rabbits with forceps and stored in vials on wet ice.

Tissues from the heart, spleen, liver and

TABLE 1. Serologic prevalence of the agents of Rocky Mountain spotted fever, borreliosis, and sylvatic plague in 76 black-tailed jack rabbits from western Texas.

Disease agent	Winter 1987				Spring 1988				Total			
	N[a]	n[b]	x̄[c]	Range[d]	N[a]	n[b]	x̄[c]	Range[d]	N[a]	n[b]	x̄[c]	Range[d]
Rickettsia rickettsii	30	10	1:294	1:128–1,024	46	11	1:326	1:128–1,024	76	21	1:311	1:128–1,024
Borrelia spp.	30	0	0	0	46	1	1:256	1:256	76	1	1:256	1:256
Yersinia pestis	30	0	0	0	46	0	0	0	76	0	0	0

[a] Number of jack rabbits sampled.
[b] Number of jack rabbits with positive antibody titers.
[c] Mean antibody titer.
[d] Range of antibody titers.

kidney were placed in separate vials containing 10% buffered formalin. All whole blood, sera, thick blood smears, and arthropod and tissue samples were tested for either disease agents, antigens and/or antibody titers against *R. rickettsii* and *Borrelia* spp. at the Texas State Department of Health (Austin, Texas 78767, USA). Sera from jack rabbits were examined for complement-fixing antibodies against *R. rickettsii* (Lane et al., 1981) and against antibodies to *Borrelia* spp. by indirect immunofluorescence (Russell et al., 1984). Serum antibody titers were considered significant at ≥1:128 for *R. rickettsii* and *Borrelia* spp. Ticks were inspected for rickettsiae and spirochetes by direct immunofluorescence of individually dissected midgut tissue (Burgdorfer and Lackman, 1960; Lane and Burgdorfer, 1988) and for rickettsial pathogens by the hemolymph test (Burgdorfer, 1970). Attempts to identify rickettsiae from tissue were made by indirect fluorescent antibody tests (Lane et al., 1981). Attempts to isolate spirochetes were made from whole blood and tissue inoculated into BSK II medium (Barbour, 1984; Lane and Burgdorfer, 1988), and to identify spirochetes on thick blood smears by direct immunofluorescence (Lane and Burgdorfer, 1988). Nobuto Strip Type I papers were sent to Plague Branch, Centers for Disease Control (Fort Collins, Colorado 80522, USA) and tested for antibodies against *Y. pestis*. Each Nobuto paper was cut into three to four parts and soaked in phosphate-buffered solution (pH = 7.2) for 60 min at room temperature to extract the antibody components; the suspensions were tested by a passive hemagglutination test (Wolff and Hudson, 1974).

Of the 76 jack rabbits tested, 28 and 1% showed positive antibody titers to the agents of RMSF and borreliosis, respectively (Table 1). Percent antibody titers to RMSF and *Borrelia* spp. were 33 and 0%, respectively, in jack rabbits captured in winter; these values were 24 and 2%, respectively, in jack rabbits captured in

spring. Only one jack rabbit had positive antibody titers to the agents of both RMSF and borreliosis.

Spirochetes or rickettsiae were not isolated from any of the tissue or blood specimens. Plague antibodies were not detected in any of the 76 jack rabbits tested.

Ticks or fleas were not found on the 30 jack rabbits captured in December 1987; however, 18 ticks were recovered from 12 of 46 jack rabbits obtained in spring 1988, including 7 *Haemaphysalis leporispalustris* and 11 *Dermacentor parumapertus*. Of these ticks, four flat *D. parumapertus* (22%) tested positive by the fluorescent antibody test for the agent of RMSF. Rickettsiae were not detected from any of the ticks by the hemolymph test (Burgdorfer, 1970).

Although rickettsiae were not isolated from the tissue or blood samples, the high serum antibody titers suggested that the jack rabbits had been exposed to *R. rickettsii*. Positive RMSF antibody titers were reported in the sera of 26 of 542 black-tailed jack rabbits in rural Kansas (Pagan et al., 1961) and in 37 of 135 black-tailed jack rabbits in California (Lane et al., 1981). Such a large percentage of jack rabbits with antibodies to *R. rickettsii* in an urban environment such as Lubbock, Texas (human population >200,000) has not been documented previously. An urban outbreak of RMSF was reported in 1987 when four persons contracted the disease in city parks in the Bronx, New York (USA) (Salgo et al., 1988).

Dermacentor parumapertus can transmit virulent laboratory strains of *R. rickettsii* (Parker et al., 1933). Due to the overlap of distributions of *Dermacentor variabilis* and *Amblyomma americanum*, ticks more commonly found on humans (Harwood and James, 1979), with *D. parumapertus* more commonly found on rabbits (Parker et al., 1939; Philip et al., 1955), there is the possibility of cross-transmission from black-tailed jack rabbits which are hosts for both species of these ticks. Rocky Mountain spotted fever was diagnosed in a woman who resided in Lubbock, Texas in 1985; the tick was possibly contracted from her pet dog (J. R. Johnson, unpubl. data). On 30 April 1985 and 5 July 1987 brown dog ticks (*Rhipicephalus sanguineus*) removed from dogs from Lubbock were fluorescent antibody (FA) positive for RMSF (J. R. Johnson, unpubl. data). In the present study the four ticks that tested positive to RMSF were flat and located on four different jack rabbits. However, only one of these jack rabbits showed a positive antibody titer to *R. rickettsii* which suggested that these ticks may not yet have infected the jack rabbits or that the antibody titers in the jack rabbits were not yet detectable. Also, it should be emphasized that our collections were made during periods of relatively low tick prevalence (winter and spring versus late summer and fall when ticks are more abundant in western Texas; D. B. Pence, unpubl. data). Thus, our data on the prevalence of *R. rickettsii* in jack rabbits in western Texas may be conservative.

The one IFA titer of 1:256 to *Borrelia* spp. could be reflective of the agent of borreliosis; alternatively, it could be a false positive reaction, due to other spirochetes, or a non-specific reaction. Regardless, the low prevalence of antibodies to *Borrelia* spp. in this locality may result from the absence of the normal vectors, *Ixodes dammini* or *I. pacificus* (Burgdorfer et al., 1985; Steere et al., 1978; Wallis et al., 1978; Westrom et al., 1985), and the absence of deer (*Odocoileus* spp.) from our study area. Thus, it is doubtful that the antibodies that were detected against *Borrelia* spp. from jack rabbits in western Texas were actually *B. burgdorferi* and it is doubtful that the Lyme disease spirochete is present in this study area.

Although rabbits have been recorded as reservoir hosts for *Y. pestis* and sylvatic plague is present in western Texas (Kartman et al., 1966), none of the jack rabbits was positive for antibodies to *Y. pestis*. Two possible explanations for the lack of plague antibodies in jack rabbits are that

(1) jack rabbits may not be reservoir hosts for the appropriate flea vectors, or (2) jack rabbits die quickly once exposed to the disease. The latter is observed in black-tailed jack rabbits that have been exposed to *Francisella tularensis*, the causative agent for tularemia (Philip et al., 1955), thus rendering serological detection of this infection in wild populations difficult. Although reported from counties to the west, northwest and southwest, sylvatic plague is presently unknown from the study site in Lubbock County in the natural host, the prairie dog (*Cynomys ludovicianus*).

The results of this study emphasize the potential importance of a common species of urban wildlife in the western United States as a reservoir and as a host for the vectors of certain important zoonotic diseases. Undoubtedly, the black-tailed jack rabbit may be a reservoir for additional zoonoses in this locality.

We thank W. W. Rossier of the Texas Department of Health for borreliosis and RMSF testing; T. J. Quan of CDC in Fort Collins for sylvatic plague testing; and the assistance of S. L. Henke, M. Pollock, T. Frieman, D. Lister, D. Osborn, P. Chubb, L. Hillock, N. VanPelt and A. Hartsell in collecting the jack rabbits examined in this study. The junior author (DBP) expresses appreciation to Donald J. Forrester who assumed responsibility as Acting Editor for the Journal of Wildlife Diseases for the review of this paper, including its decision on acceptance or rejection.

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Received for publication 17 January 1989.